REMARKS

Claims 1, 2, 4-8 and 12-16 are currently pending. A "clean set" of the amended claims is provided above. Indication of the amendments made is provided below. Applicants submit that the amendments raise no new issues that would require further search and respectfully request these amendments be entered.

Claims 1, 4 and 15 have been amended for clarity. Support is found, for example, at page 8, line 6 and at page 11, lines 3-11.

Claim 8 has been amended to specify that the cells are prepared using antibody binding of RET and to specify the types of cells in the population. Support is found, for example, at page 8, line 6 and at page 11, lines 3-11.

Rejection Under 35 U.S.C. § 112

Claims 1, 2, 4-7 and 15 are rejected under 35 U.S.C. § 112, second paragraph for reciting a phrase that lacks antecedent basis. Applicants respectfully traverse.

Applicants submit that a RET protein inherently has a sequence. Therefore, "the RET sequence" should not require antecedence. However, for the sake of clarity, Claims 1, 4 and 15 have been amended to clarify that the antibody specifically binds a sequence of the RET protein.

Applicants submit that Claims 1, 2, 4-7 and 15 satisfy the requirements of 35 U.S.C. § 112, second paragraph. Therefore, withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. § 102

Claims 8 and 16, and Claims 13 and 14, per their dependence from Claim 16, are rejected under 35 U.S.C. § 102(b) as being anticipated by Stemple et al., *Cell* 71:973-985 (1992) (Stemple I). Applicants respectfully traverse.

To anticipate a claim under 35 U.S.C. § 102, each element of the claim must be taught or suggested in a single prior art reference. Claim 8, as amended, is directed to a substantially pure population of neural crest derived neural progenitor cells prepared by antibody binding of RET. Claim 16 is directed to a substantially pure population of neural

crest derived neural progenitor cells comprising RET protein. Neither of these populations is disclosed or suggested in Stemple I.

Stemple I discloses isolating neural crest cells with an antibody to the low affinity nerve growth factor (LNGFR). As the specification points out, LNGFR is a neural crest stem cell (NCSC) marker, which cells do not express RET antigen (*see* page 21, lines 12-14 of the specification). The present disclosure investigated and compared these populations of cells (*see* page 26, lines 8-20). These two antibodies isolated disparate populations both in number (up to 10 times more cells labeled by anti-LNGFR) and character (6.5% LNGFR+ cells were NP versus 50% of RET+ cells). From these experiments, it is clear that a population of neural crest derived cells isolated using an antibody to LNGFR is not the same population as that presently claimed.

For the reasons discussed above, Claims 8, 16 and 13 and 14 are not anticipated by Stemple I. Therefore, Applicants respectfully request that this 35 U.S.C. § 102(b) rejection be withdrawn.

Rejection Under 35 U.S.C. § 103

Claims 1, 2, 4-8 and 12-16 are rejected under 35 U.S.C. § 103(a) as being obvious over Lo et al., *Perspectives Dev. Neurobiol.* 2:191-201 (1994) (Lo), Stemple *et al.*, *Dev. Biol.* 159:12-23 (1993) (Stemple II), Stemple *et al.*, *Cell* 71:973-985 (1992.) (Stemple I), and Martucciello *et al.*, *J. Ped. Surg.* 30(3):433-436 (1995) (Martucciello). Applicants respectfully traverse.

For a rejection under 35 U.S.C. § 103 to be proper, it must be shown that: 1) each element of a claim is disclosed or suggested in the prior art; 2) the prior art provided motivation to combine and/or modify prior art disclosures to obtain the claimed invention; and 3) the skilled artisan would have a reasonable expectation of successfully obtaining the claimed invention. Applicants submit that all of these have not been met in the present rejection.

Lo discloses expression of *MASH-1* and *c-ret* mRNA in developing sympathetic and enteric nervous tissue and adrenal gland. This reference provides no evidence of expression of the protein, nor does it discuss whether the protein is expressed in developing

tissue. The Office Action mailed 4/1/98 (the only Office Action making any specific reference to the cited art itself), states that Lo teaches RET as a valuable marker for early stages of neural crest cell lineage diversification. Applicants point out that Lo refer only to the *c-ret* gene as a marker, not the protein, and provide no suggestion as to how cells expressing this gene might be isolated.

Stemple II provides a review of the influences on developing cells and references the various approaches that have been taken in such study. While this reference does describe uses of antibodies in this research, Stemple II does not mention RET protein. Furthermore, Stemple II provides a caveat for those using an antibody approach, noting that "antigenic heterogeneity in neural crest cell populations cannot necessarily be taken as evidence of heterogeneity in developmental potential." (Page 17, right column, first full paragraph).

Stemple I discloses isolating neural crest cells with an antibody to the low affinity nerve growth factor (LNGFR). This reference makes it very evident that the use of this technique was based on the well-established knowledge that the LNGFR protein is not only expressed in these cells, but is expressed at the cell surface. Furthermore, Stemple I makes it clear that the use of this technique was based on the availability of antibodies known to bind to the extracellular portion of this protein rat neural crest cells.

Martucciello describes antibody labeling of RET protein in diseased and normal adult human tissue . This reference describes RET as a protein expressed in human tumors. This reference provides no evidence of the mode or even the fact of expression of this protein in developing cells.

Claims 1 and 2 are directed to a composition comprising a monoclonal antibody and a cell selected from the group consisting of a multipotent neuronal progenitor (proNP) cell, a nonneuronal progenitor (NNP) cell and a committed neuronal progenitor (NP) cell, each of which comprise RET protein. The antibody is specifically bound to a sequence of the RET protein. Claims 4-7 and 15 are directed to a method for enrichment of neural progenitor cells comprising RET protein. The method comprises combining neural crest derived cells comprising neural progenitor cells with an antibody and isolating RET

positive cells. Claims 8-14 and 16 are directed to a substantially pure population of neural crest derived neural progenitor cells prepared using antibody binding to RET protein.

All Elements Not Taught

In all of the present claims, there is the element of a neural progenitor cell comprising RET protein such that an antibody specific thereto can bind the protein. None of the cited references, alone or in any combination, teach neural crest cells expressing RET protein. Nor do any of these references teach expression of RET protein in neural crest cells such that it may be bound by an antibody. Prior to the present disclosure, it was not known that developing neural crest cells expressed the RET protein, nor that the RET protein was expressed at the cell surface, nor that the RET protein presented an antigen that could be bound by an antibody on the cell surface and used to perform the methods and provide the compositions and cell populations of the present claims.

In addition, none of the cited references teach neural progenitor cells, a distinct and specific subset of neural crest derived cells, which express RET protein, or express the protein such that an antibody can bind at the cell surface so that it may be used to enrich a population of cells with neural progenitor cells or to prepare a substantially pure population of neural progenitor cells.

The lack of disclosure of these claim elements has been brought up before in the prosecution history of this application and has not been addressed in subsequent Office Actions. The criteria to support a *prima facie* case for obviousness are specific and all of these criteria must be met. Repeated insistence that the skilled artisan would be motivated to combine references does not make up for the shortcomings of the references with regard to the other two criteria.

No Motivation to Combine or Modify Prior Art to Provide the Present Invention

The Office Action mailed April 1, 1998 suggests that it would be obvious to use antibodies such as disclosed in Martucciello for immunological fractionation of RET+ cells by conventional methods such as disclosed in Stemple I because Stemple II says such methods have been useful and Lo teaches that RET is a useful marker for early neural crest

cell lineage (page 12 of Office Action mailed 4/1/98). However, this combination of references lacks the critical disclosure to motivate the ordinary skilled artisan to make such modifications so as to obtain the present invention. Without the knowledge that RET protein is in fact expressed in developing neural crest cells and that antibodies could be obtained to label neural cells, the skilled artisan would not be motivated to obtain the present invention. Furthermore, none of the references teach or suggest that neural progenitor cells express RET protein so as to provide motivation to obtain the present invention.

The Office Action analysis takes a giant leap past the absence of disclosure that developing neural crest cells express RET protein or that such RET protein, if expressed, could be labeled by antibodies. Furthermore, the Office Action takes a second leap to suggest that such labeling would obviously allow for the preparation of a composition or population of cells comprising neural progenitor cells. The combination of references cited provides for the investigation of the lineage of RET protein-expressing cells in tumors and Hirschsprung's disease, not the present invention.

No Reasonable Expectation of Success

As discussed above, the lack of information regarding RET in neural crest derived neural progenitor cells left inherent uncertainties at the time of invention as to whether these cells expressed the RET protein or whether , even if they did, that it would be sufficiently antigenic to allow antibodies to specifically bind so as to permit enrichment of such cells. With this inherent uncertainty, the ordinary skilled artisan would not have a reasonable expectation that such methods for enrichment, or compositions or populations of cells as found in the present claims, would result, even if the steps of the methods were tried. This last fact is further supported by the statements in Stemple II, warning that immunogenic heterogeneity does not necessarily suggest heterogeneity in developmental potential. The neural progenitor cells of the present claims have a distinct developmental potential that could not be predicted to be different from, for example, LNGFR expressing cells, even if it was known that neural crest cells did express RET protein in the manner necessary to obtain the present invention.

For the reasons discussed above, Claims 1, 2, 4-8, and 12-16 are not obvious over Lo, Stemple II, Stemple I and Martuciello. Therefore, Applicants respectfully request that this 35 U.S.C. § 103(a) rejection be withdrawn.

CONCLUSION

Applicants respectfully submit that the claims are now in condition for allowance and an early notification of such is solicited. If the Examiner believes a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned attorney.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Four Times Amended) A composition comprising a monoclonal antibody and a cell selected from the group consisting of a multipotent neuronal progenitor (proNP) cell, a nonneuronal progenitor (NNP) cell and a committed neuronal progenitor (NP) cell, each of which comprise RET protein, wherein said monoclonal antibody is specifically bound to all [of] or part of [the] a sequence of said RET protein [sequence] on said cell.

2. (Twice Amended) The composition according to claim 1, wherein said sequence consists essentially of the extracellular domain of RET.

4. (Four Times Amended) A method for the enrichment of neural progenitor cells comprising RET protein, said method comprising:

a) combining a mixed population of cells comprising neural-crest derived cells comprising neural progenitor cells with an antibody that specifically binds to all of part of [the] a sequence of said RET protein [sequence]; and

b) selecting for RET positive cells, whereby the percentage of neural progenitor cells is enriched.

5. (Amended) The method according to claim 4 wherein said antibody is selected from the group consisting of polyclonal antibody, monoclonal antibody, antibody fragments, and single chain antibody.

6. (Amended) The method according to claim 5, wherein said antibody is fluorochrome conjugated.

7. (Twice Amended) A method according to claim 6, wherein said selecting with said fluorochrome conjugated antibody is by flow cytometry.

8. (Four Times Amended) [A substantially pure population of neural crest derived neural progenitor cells prepared using antibody binding, where] The population according to Claim 16, wherein said cells are nonneuronal progenitor (NNP) cells.

12. (Twice Amended) The population according to claim [8] 16 wherein said neural progenitor cells are bound to an antibody that specifically binds to RET antigen.

13. (Twice Amended) The population according to claim 12 or 16 wherein said antibody is selected from the group consisting of polyclonal antibody, monoclonal antibody, antibody fragments, and single chain antibody.

14. (Amended) The population according to claim 13 wherein said antibody is a monoclonal antibody.

15. (Thrice Amended) A method for the enrichment of neural progenitor cells, said method comprising:

a) combining a mixed population of cells comprising neural-crest derived cells comprising neural progenitor cells comprising RET protein with a monoclonal antibody that specifically binds to all of part of [the] a sequence of said RET protein [sequence]; and

b) selecting for RET positive cells, whereby the percentage of neural progenitor cells is enriched.

16. (Amended) A substantially pure population of neural crest derived neural progenitor cells comprising RET protein prepared using antibody binding to RET protein, where said cells are proneuronal progenitor (proNP) cells, neuronal progenitor (NP) cells and/or nonneuronal progenitor (NNP) cells.